ANTIMICROBIAL RESISTANCE AND RESISTANCE GENES IN *STAPHYLOCOCCUS AUREUS* STRAINS FROM RABBITS

VANCRAEYNEST D.[1], HERMANS K.[1], MARTEL A.[1], VANEECHOUTTE M.[2], DEVRIESE L.A.[1], HAESEBROUCK F.[1]

[1] Department of Pathology, Bacteriology and Poultry Diseases, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium.
[2]Department of Clinical Chemistry, Microbiology and Immunology, Faculty of Medicine, Ghent University, De Pintelaan 185, B-9000 Ghent, Belgium.
dieter.vancraeynest@UGent.be

ABSTRACT

Fifty-six *Staphylococcus aureus* isolates recovered between 1998 and 2003 from 31 rabbit farms with and without problems of chronic staphylococcosis, were screened for resistance to enrofloxacin, erythromycin, gentamicin, lincomycin, neomycin, penicillin and tetracyclines using the agar dilution test. For penicillin, a disk diffusion test was also performed. The detection of *tet*P(B), *tet*(K), *tet*(L), *tet*(M), *tet*(O), *tet*(T), *tet*(W), *erm*(A), *erm*(B) and *erm*(C) genes was done via a PCR assay. Four isolates showed resistance to erythromycin and lincomycin. These isolates were positive in the PCR assay for the *erm*(C) gene which encodes resistance to lincosamides, macrolides and streptogramine B antibiotics (LECLERCQ and COURVALIN, 1991). Eleven strains were resistant to tetracyclines and all were positive for the *tet*(K) gene encoding active efflux of tetracyclines (HIRATA *et al.*, 1998). In the agar dilution test, five isolates showed resistance to penicillin, whereas in the disk diffusion test 12 isolates showed resistance. Only one strain showed resistance to gentamicin, and all strains were susceptible to enrofloxacin and neomycin. This study demonstrates that resistance to antimicrobial agents in *S. aureus* isolates originating from rabbits is relatively rare compared to resistance in *S. aureus* isolates originating from other animals and from humans.

Key words: *Staphylococcus aureus*, rabbit, resistance, antimicrobial agents.

INTRODUCTION

In individual rabbits, all *S. aureus* infections have a similar clinical appearance, with lesions of pododermatitis, subcutaneous abscesses and mastitis (OKERMAN *et al.*, 1984; HOLLIMAN and GIRVAN, 1986; ROSSI *et al.*, 1995; DEVRIESE *et al.*, 1996). Sometimes internal organ abscesses are observed as well, predominantly in lungs, liver and uterus. This leads to poor production results, infertility and death. Suckling young may die as a result of agalactia in the doe. At rabbit flock level, two types of *S. aureus* infections can be distinguished. In the first type, caused by low virulence strains, the infection remains limited to a small number of animals. This type only has a minor economic importance. The second type of infection is caused by the high virulence strains, which spread throughout the rabbitry. This leads to chronic problems.

It is generally accepted that infections with high virulence *S. aureus* strains in rabbits cannot be eradicated with antimicrobial agents (OKERMAN et al., 1984; CAROLAN, 1986; HOLLIMAN and GIRVAN, 1986; ROSSI *et al.*, 1995). The reason for this remains unknown. Little information is available on the antimicrobial resistance of *S. aureus* strains from rabbits. The purpose of this study was to determine the prevalence of resistance phenotypes for a number of antimicrobials among rabbit *S. aureus* isolates and to screen for the genes responsible for their resistance.

MATERIALS AND METHODS

Bacterial isolates

Fifty-six rabbit *S. aureus* strains were tested. Nineteen of these strains were high virulence strains, all isolated from commercial rabbitries with chronic problems of staphylococcosis. Eighteen of these strains belonged to the biotype – phage type combination “mixed CV-C” – 3A/3C/55/71. One strain belonged to the biotype – phage type combination “mixed CV-C” – 29/79/42E/92/D11/HK2 (DEVRIESE *et al.*, 1996). The other strains were low virulence strains, belonging to other biotype – phage type combinations.

Dilution susceptibility testing

Minimal Inhibitory Concentrations (MICs) of the following antimicrobial agents were determined: enrofloxacin, erythromycin, gentamicin, lincomycin, neomycin, penicillin and tetracyclines. The MICs were determined using the NCCLS (National Committee for Clinical Laboratory Standards) agar dilution method (NCCLS, 1999), except for the fact that Brain Heart Infusion broth (Oxoid, Basingstoke, England) was used to grow the inocula. After overnight incubation at 37°C, these inocula were suspended in 0.9% NaCl solution to a 0.5 McFarland standard. By means of a Steers inoculum applicator, a 1/10 dilution of these suspensions was inoculated on Mueller-Hinton II agar (Beckton Dickinson, Le Pont de Claix, France) containing erythromycin, gentamicin, lincomycin, neomycin and tetracycline concentrations ranging from 0.12 µg/ml to 64 µg/ml and enrofloxacin and penicillin concentrations ranging from 0.03 µg/ml to 8 µg/ml. The plates were incubated at 37°C and observed after 24h. The MIC was defined as the lowest concentration producing no visible growth. Criteria for resistance to enrofloxacin, erythromycin, gentamicin, penicillin and tetracyclines were those formulated in the NCCLS Approved Standard (NCCLS, 1999). To evaluate resistance to lincomycin and neomycin, a microbiological criterium was used: strains exhibiting visible growth at concentrations of lincomycin and neomycin four times higher than the concentration inhibiting a sensitive reference strain *S. aureus* ATCC 29213 were considered resistant.

Disk susceptibility testing

To detect ß-lactamase (penicillinase) activity, penicillin G susceptibility tests were additionally determined by disk diffusion on Isosensitest agar (Oxoid, Basingstoke,

England) using Penicillin Low Neo-Sensitabs (Rosco Diagnostics, Taastrup, Denmark). The plates were inoculated with the above described 0.5 McFarland standard suspension and after overnight incubation at 37°C, zone edges were examined. ß-lactamase positive strains show heaped-up borders, while inhibition zones of susceptible strains have diffuse margins.

PCR assay on antibiotic resistance genes

A PCR assay was performed to detect the presence of *tet*(K), *tet*(L) (TRZCINSKI *et al.*, 2000), *tet*(M), *tet*(O), *tet*P(B), *tet*(T), *tet*(W) (AMINOV *et al.*, 2001), *erm*(A), *erm*(B) and *erm*(C) (SUTCLIFFE *et al.*, 1996) in all isolates.

To prepare DNA, one colony of bacterial cells was suspended in 20 µl lysis buffer (0.25% SDS, 0.05N NaOH) and heated at 95°C for 5 minutes. The cell lysate was spinned down by short centrifugation, and then diluted by adding 180 µl distilled water. Another centrifugation for 5 minutes at 16000 g was performed to remove the cell debris. Supernatants were frozen at -20°C until further use.

After amplification electrophoresis was performed. Gels were visualized under U.V. light and photographed.

RESULTS AND DISCUSSION

Susceptibility testing

A total of 54% of the tested rabbit *S. aureus* strains were susceptible to all seven antimicrobial agents tested.

The highest frequency of resistance in the agar dilution test was found for tetracyclines (20%). There is a striking difference between the level of resistance of the rabbit isolates to tetracyclines (20%) and the level of resistance (>90%) formerly described in poultry isolates (GEORNARAS and VON HOLY, 2001).

In the agar dilution test, only five strains were resistant to penicillin. However, with penicillin susceptible to staphylococcal ß-lactamases, interpretation of MIC-tests is difficult. The very small bacterial inocula used in the NCCLS procedure do not allow sufficient production of this enzyme to become easily detectable. Therefore the disk diffusion test was performed as well. In this test, twelve of the 56 strains (21%) were found to produce ß-lactamase. This frequency of penicillin resistance is low compared to levels described for human (over 90%) as well as bovine (around 50%) isolates (HAMMOND and NORRISS, 1995; DEVRIESE *et al.*, 1997; WERCKENTHIN *et al.*, 2001). Penicillin therapy in rabbits often leads to dysbiosis, enteritis, enterotoxaemia and finally death (BURGMANN, 2000). Because of these restrictions in its use, penicillin selection pressure on *S. aureus* strains from rabbits is probably lower compared to other animal species and humans. This may explain the relatively low percentage of penicillin resistance found in this study.

Four strains were resistant to both erythromycin and lincomycin and one strain showed resistance to both penicillin and gentamicin. All strains were susceptible to neomycin and enrofloxacin. The levels of resistance to erythromycin, lincomycin and neomycin are comparable to those found in bovine isolates (DEVRIESE *et al.*, 1997; WERCKENTHIN *et al.*, 2001). Enrofloxacin and gentamicin resistance is in agreement with the situation for bovine *S. aureus* isolates as well, except for the frequencies of resistance in Germany, where resistance for bovine *S. aureus* isolates is 10.1% for enrofloxacin and 51.2% for gentamicin (WERCKENTHIN *et al.*, 2001).

The limited use of certain antimicrobial agents in rabbits because of their toxic effects is not the only factor causing a relatively low selection pressure on rabbit *S. aureus* strains. As rabbit abscesses tend to be walled off and caseous, they are relatively inaccessible to antimicrobial agents (BURGMANN, 2000). In that way, *S. aureus* bacteria might be less affected by antimicrobial selection pressure as well. This may explain the rather low antimicrobial resistance in rabbit *S. aureus* strains.

PCR assay on antibiotic resistance genes

The four strains resistant to erythromycin and lincomycin were *erm*(C) positive in the PCR assay. This gene encodes resistance to lincosamides, macrolides and streptogramine B antibiotics (LECLERQ and COURVALIN, 1991). A possible explanation for the occurrence of the *erm*(C) gene among rabbit *S. aureus* isolates is the use of spiramycine in rabbits. None of the strains carried the *erm*(A) or the *erm*(B) gene.

All of the strains resistant to tetracyclines were *tet*(K) positive in the PCR assay. None of the strains were positive in the PCR assay for *tet*P(B), *tet*(L), *tet*(M), *tet*(O), *tet*(T), and *tet*(W).

CONCLUSIONS

In view of the fact that antibiotic resistance percentages are low in rabbit *S. aureus* strains, we can conclude that there have to be other reasons why antibiotic therapy is failing in eradicating high virulence *S. aureus* strains at rabbit flock level. Hypotheses about the reason of persistence include the existence of carrier rabbits and the survival of the bacterium in the environment. However, the exact cause remains to be elucidated.

ACKNOWLEDGEMENTS

This work was supported by a Ph.D. grant of the Institute for the Promotion of Innovation through Science and technology in Flanders (IWT Vlaanderen) to Dieter Vancraeynest. Arlette Van de Kerckhove is acknowledged for her skilled technical assistance.

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